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Comparison of the Growth of Gynogenetic Diploids with Control Diploids in Hirame *Paralichthys olivaceus* Raered in the Same Tank

Kazuo Tabata*2 and Shigeaki Gorie*2

The growth of gynogenetic diploids was compared with that of control diploids of hirame *Paralichthys olivaceus* by rearing in the same tank from larval to young stage. *Idh-1* isozyme was used as genetic marker to distinguish each group in the mixed population.

The average body weight of gynogenetic diploids was significantly lower than control diploids. It was assumed that the differences of growth observed in this experiment were caused by endogenous factor because both groups were exposed to the same environmental conditions.

It is an interesting fact from the point of view of breeding that the coefficient of variations of body weight in gynogenetic diploids were larger than control diploids.

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*1 Studies on the Gynogenesis in Hirame Paralichthys olivaceus - VI. Nakazaki,

*2 Hyogo Prefectural Fisheries Experimental Station, Akashi, Hyogo 673, Japan.

Induced production of gynogenetic diploids has already been attempted with numerous fish species and its conditions too have practically been elucidated [1-4]. However, there are practically no fish species regarding which one knows the states of growth and survival following induced production. The present authors have already clarified the conditions of induced production of gynogenetic diploids for hirame Paralichtys olivaceus*[4]), the proofs of induced production [5]), the methods of effective induced production [6], [7]) and such, and they have reached the stage at which a detailed knowledge of the growth and survival rate is required. With the purpose of comparing the rearing characteristics of gynogenetic diploids and of normally produced diploids, the authors performed rearing tests in separate water tanks but as the growth results diferred each time they performed rearing, they were unable to clarify any superiority (unpublished). Differences in growth are largely determined by environmental conditions such as rearing density, feed density or temperature. Keeping such environment-dependent conditions strictly constant is extremely difficult and more particularly at the early rearing stage. There are thus such limitations to uniformity with separate rearing. However, since the effect of the environment on the two groups would be completely the same if one could rear the two groups simultaneously in the same water tank, one could estimate that the obtained differences in growth are caused by endogenous factors [8-10]). On the basis of this concept, we performed comparative rearing by holding gynogenetic diploid and normally produced flounder eggs in the same water tank, and gained some knowledge regarding their

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* translator's note: referred to hereafter as "flounder"

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growth and survival. This is reported hereunder.

Materials and methods

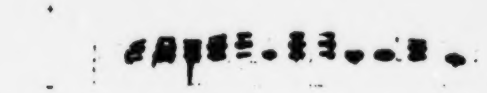
Determining the genotype of the parent fish and of the fry

In order to be able to distinguish the two different groups held in the same water tank, we used genetic marking based on isozymes. First, we examined by biopsy the genotypes of the Idh-1 isozymes (isocitrate dehydrogenase, E.C. 1.1.1. 42) of the parent flounder, and choose as parent fish a AA-type male and BB-type females. The multiplicity of isozyme types is generally explained by co-dominant alleles. For the Idh-1 isozymes of flounder too, direct explanation is given through mating tests [11]). Consequently, the genotype of the gynogenetic diploids is BB while that of the normally produced specimens (hereafter "the control group") is AB. The two groups can be recognized if one examines the isozymes.

Induced production of gynogenetic diploids and rearing methods

We performed rearing tests twice (tests 1,2) and used one female and one male each for the respective tests. Different females were used for the tests 1 and 2. The male was the same for both tests.

We divided the collected eggs into 2 groups of 10,0 g (14,900 eggs). After inseminating by the usual method [4]) one group with spermatozoids genetically inactivated through UV rays (4,800 erg/mm^2), we immersed (the eggs) for 45 min in 0° seawater and obtained gynogenetic diploids. We inseminated the other group of eggs with untreated spermatozoids.



Genotype AB AB AB AB AB BB AB AB BB BB AB BB

Fig. 1. Expamples of *Idh-1* isozyme in gynogenetic diploids (*BB* type) and control diploids (*AB* type) of hirame *Paralichthys olivaceus* reared in the same tank. This zymogram was detected from samples of larvae 30 days after hatching in experiment 2.

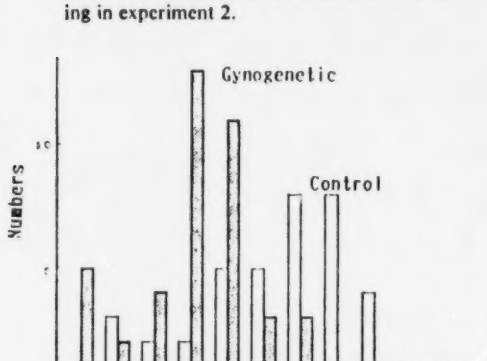


Fig. 2. Frequency of the body weight of gynogenetic diploids and control diploids in hirame *Paralichthys olivaceus* reared in the same tank (experiment 1) (30 days after hatching).

Table 1. Comparison of the average body weight between gynogenetic diploids (Gyno-2n) and control diploids (Cont.) in hirame *Paralichthys olivaceus* reared in the same tank

	Days after hatching	Genotype of *Idh-1* isozyme	Number sampled	Gyno-2n. or Cont.	Ave. body weight ±stand. deviation	Coefficient of variations
Exp. 1						
	30	*BB*	41	Gyno-2n	21.3±4.89 (mg)	.229
		AB	39	Cont.	28.2±6.55	.232
Exp. 2						
	26	*BB*	14	Gyno-2n	13.6±2.03 (mg)	.149
		AB	46	Cont.	13.7±4.34	.317
	39	*BB*	42	Gyno-2n	60.6±18.95 (mg)	.316
		AB	58	Cont.	73.6±18.44	.251
	221	*BB*	32	Gyno-2n (Female)	164.1±53.08 (g)	.324
		BB	6	Gyno-2n (Male)	132.8±33.50	.252
		BB	38	Gyno-2n (F.+M.)	159.1±51.50	.324
		AB	23	Cont. (Female)	234.0±58.99	.252
		AB	17	Cont. (Male)	206.0±39.74	.193
		AB	40	Cont. (F.+M.)	222.1±52.99	.239

After culturing for 2 days in separate tanks at the water temperature of 16.5 - 17.2°C, we put equal amounts of ready-to-hatch eggs from both groups (440 individuals each in test 1 and 1400 each in test 2) in one water tank (100 L). After the hatching, we used the feed described hereunder and reared in running seawater; Brachionus plicatilis (2 - 35 days after hatching), Artemia salina (10 - 45 days after hatching), ami shrimps(41 - 50 days) and sand launce (51 - 220 days after hatching). To rear, we kept the fry in a 100 L water tank up to 39 days after hatching, then from the 40th day inside a 200 L net crawl installed in a 500 L water tank. After 110 days we left the fish free and unhindered inside the 500 L water tank. p. 1144

In the test 1, we sampled the fry at random on the 30th day following hatching, and did the same in test 2 on the 26th, 39th and 221st days. After measuring the total lengths and the weights, we examined the Idh isozymes in the tissues including the liver. The Idh-1 genetic loci are detected in the liver and the Idh-2 genetic loci are detected in the muscle tissues. However, we excluded the Idh-2 genetic loci as they are of a single type.

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Isozyme analysis

We shredded with scissors the entire frozen bodies of flounder samples on the 26th and 30th days after hatching, crushed them further with the tips of pincettes and used this as crude enzyme solution. Using electronic microscope pincettes under a stereoscopic microscope, we prepared a similar solution from flounder 39 days after hatching. On the

221st day we extracted directly the liver of the flounders, froze them for a while and used the exudate following thawing as crude enzyme solution.

The isozyme analysis was carried out by the horizontal starch gel electrophoresis method (gel concentration: 12%; electricity circulated at the rate of 4 mA per 1 cm^2 gel section) [5].

Results

Results of isozyme analysis

We were able to obtain clear electrophoretic images from flounders hatched for 26 and 30 days. The entire bodies had been used as sample. Fig. 1 shows an example of Idh-1 isozyme analysis.

Since the parent's genotype is BB for the female and AA for the male, all genotype of the offsprings are AB in the case of regular mating (control group). However, all genotypes of the gynogenetic diploids are BB. p. 1144

Growth comparison

Table 1 shows by number of days following hatching the compared weights in the gynogenetic diploid group and in the control group. Fig. 2 and 3 show the weight frequency distributions.

Table 2 shows the results obtained by examining the differences in

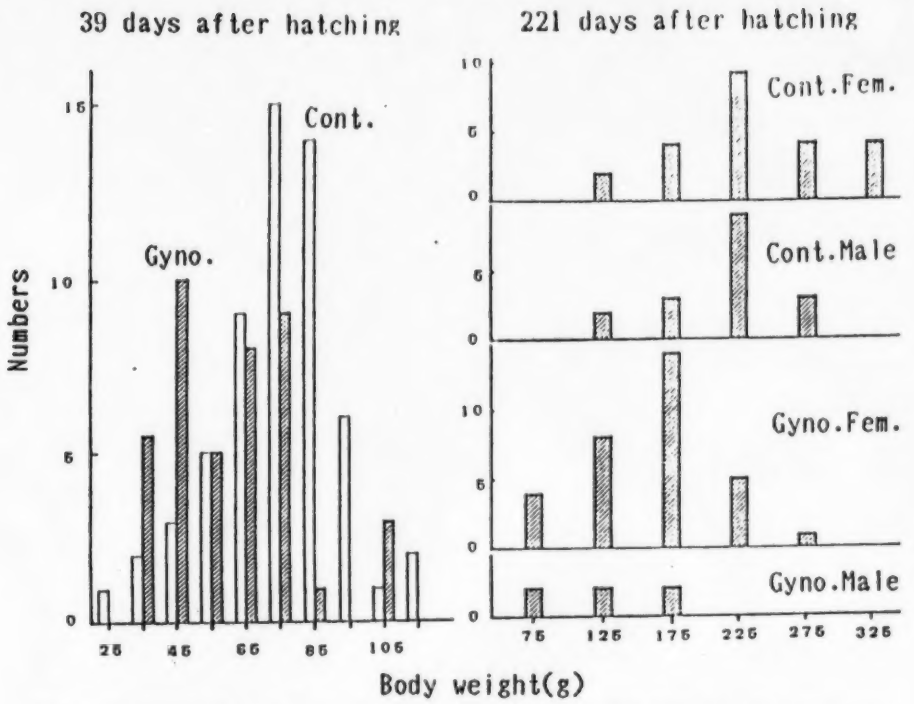

Fig. 3. Frequency of the body weight of gynogenetic diploids and control diploids in hirame *Paralichthys olivaceus* reared in the same tank (experiment 2) (39 and 221 days after hatching).

Table 2. Statistical tests of the differences of average body weight between gynogenetic diploids and control diploids in hirame *Paralichthys olivaceus* reared in the same tank

Exp. 1	
30 days after hatching	Control (*AB* type)>Gyno. (*BB* type) $t=5.53$ ($p<0.01$)
Exp. 2	
26 days	Control (*AB* type)=Gyno. (*BB* type) $t=0.12$ (NS)
39 days	Control (*AB* type)>Gyno. (*BB* type) $t=3.58$ ($p<0.01$)
221 days	Control (*AB* type, Fem.+Male)>Gyno. (*BB* type, Fem.+Male) $t=5.32$ ($p<0.01$)

NS: No significance $p<0.01$: Significance with 1% risk.

Table 3. Statistical tests of the differences of average body weight among female and male of gynogenetic dploids and control diploids in hirame *Paralichthys olivaceus* reared in the same tank (221 days after hatching, experiment 2)

		Control (*AB* type)		Gyno. (*BB* type)	
		Female	Male	Female	Male
Control	Fem.		$t=1.69$ (NS)	4.60 ($p<0.01$)	4.00 ($p<0.01$)
	Male			2.28 ($p<0.01$)	4.02 ($p<0.01$)
Gyno.	Fem.				1.38 (NS)
	Male				

NS: No significance $p<0.01$: Significance with 1% risk.

weight averages between the gynogenetic diploid group and the control group by numbers of days following hatching. Only on the 26th day following hatching in test 2 was there no significant statistical difference between the gynogenetic diploid group and the control group. On the 30th day after hatching in test 1, on the 39th and 221st days in test 2 the control group was every time superior to the gynogenetic diploid group, and one noted between the groups a statistically significant difference with a risk rate of 1%.

On the 221st day after hatching in test 2, it was possible to distinguish the males and females. We compared the respective average weights and noted no significant difference between males and females in the gynogenetic diploid group as well as in the control group. In all other combinations we noted a significant difference with a risk rate of 1% (Table 3).

The coefficient of variation tended to be greater in the gynogenetic diploid group than in the control group on the 39th and 221st days after hatching.

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Survival and sex rate

Table 4 shows the hatching rate and the survival results after hatching. In both tests 1, 2 the hatching rate of the control group was better than that of the gynogenetic diploid group. In test 1 the survival rate of the gynogenetic diploid group was 0.4% higher that that of the control group. In test 2 to the contrary the control group was superior by 4.9%.

Table 4. Comparison of hatching and survival rates of gynogenetic diploids with those of control diploids in hirame *Paralichthys olivaceus* reared in the same tank

Groups		Hatching rate	Survival rate
Exp. 1			
	Gynogenetic	15.5%	9.3%
	Control	36.4	8.9
Exp. 2			
	Gynogenetic	59.9	37.8
	Control	87.3	42.7

In the results of the examination on day 221 of test 2, the rate of female generation in the control group was 57.5% whereas it was 84.2% in the gynogenetic diploid group.

Discussion

The results of two tests in which a gynogenetic diploid group and a control group of flounders were reared in the same water tank indicated that except for the 26th day after hatching the growth of the gynogenetic diploid group was significantly inferior to that of the control group. The 26th day after hatching is the height of the transformation period and the period in which the ecology changes from free swimming to bottom sedentary life. Since from the other results of test 2 it is anticipated that the control group moves earlier to the bottom sedentary stage than the gynogenetic diploid group, one may think that maybe no random sampling was carried out.

According to our unpublished data about the rearing of a gynogenetic diploid group and of a control group in separate water tanks, the average body weight in the control group was significantly higher than in

the gynogenetic diploid group in 2 instances ($p < 0.01$). It was the opposite in 1 instance, and in 2 other instances there was no significant difference ($p > 0.05$). So, the separate rearing results are divergent. The main reason seems to be the various environmental differences inside the water tanks. The only way to achieve strict environment determination inside water tanks is to rear both groups simultaneously in the same water tank. The present differences in growth rates detected by this method should therefore be attributable to endogenous causes. If one considers that differences in growth appeared as a result of competition for feed and free space consequent to the mixed rearing of these two groups then the revealed relative physical weakness of the gynogenetic group may be considered to constitute a characteristic. The method of comparing growth and such by releasing and rearing the specimens in the same water tank and using isozyme gene tagging for groups which have different fry periods and are impossible to mark through fin notching was developed by Taniguchi et al [8-10]. We used this method here to evaluate the growth of a gynogenetic diploid group and were able to ascertain the effectivity of the method. Actually however no gynogenetic diploid group of flounders is reared together with a normally produced group, so the aforementioned results whereby the superiority or inferiority of the two groups were unclear with separate rearing may possibly be evaluated accordingly. One should increase the instances of growth-comparing tests in a situation free from multiplication* interference.

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* translator's note: or "geometrical"

Fujio et al. [12]) reported that a correlation is noted between the growth of artificial flounder seedlings and the Idh-1 isozyme genetic loci and that the heterotypes outperform the homotypes. In the present tests we established the Idh-1 genetic locus BB types (homo) for the gynogenetic diploid group, and the AB types (hetero) for the control group, so that there could still be a problem with regard to the fact that we choose a genetic locus connected to growth. Although it concerns a test totally unrelated to the present tests, Table 5 shows as example the differences in growth between homozygotes and heterozygotes in Idn-1 genetic loci of 6 test groups obtained by rearing in a virtually similar fashion groups produced through natural spawning in a pond (unpublished). The heterozygote group is certainly seen to grow better than the homozygote group but there is no statistically significant difference,and the difference was fairly small when compared to that between the gynogenetic diploid group and the control group. So, as far as these tests are concerned, one may say that there is no manifest correlation between the homo, heterozygotes of the Idh-1 genetic loci on the one hand and the growth on the other. It seems therefore that the relative growth inferiority of the gynogenetic diploid group obtained in the present tests is not directly related to the homozygous quality of the Idh-1 genetic locus. Is is more likely that because of the gynogeniticity there was an overall progress in homo-conversion and that an inbreeding depression phenomenon was seen.

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Table 5. Comparison of the average body weight between homozygotes and heterozygotes of *Idh-1* isozyme in the hatchery population of hirame *Paralichthys olivaceus* produced by natural spawning method

Groups	No.	Body weight			T-test
		Homozygotes		Heterozygotes	
86-A	40	188.5 g	<	215.0 g	1.64 (NS)
86-B	50	152.6	<	153.9	0.10 (NS)
86-C	33	128.3	<	134.4	0.42 (NS)
85-B	40	185.7	<	196.1	0.62 (NS)
85-D	40	174.8	>	171.5	0.31 (NS)
85-L	49	121.4	<	125.2	0.32 (NS)

NS: No significance

The coefficient of variation of the gynogenetic diploids tended to rise above that of the control group as the growth progressed. This might be a reflection of the degree of change of the gynogenetic diploids. These results should be interesting from the viewpoint of breeding science.

There was no clear difference between the gynogenetic diploid group and the control group with regard to survival rate based on rearing in the same water tank. The aforementioned relative inferiority of the gynogenetic diploids should not be great enough to have a relationship with life or death.

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* translator's note: reading not quite certain; same hereafter for asterisk following a personal name

雌性発生2倍体の変動係数は成長の経過とともに対照区より高くなる傾向がみられたが，雌性発生2倍体の変異の大きさを反映している可能性もあり育種学的観点から見ると興味深い結果である。

同一水槽飼育による生残率は雌性発生2倍体群と対照群の間に顕著な差はなかった。上述した雌性発生2倍体の相対的劣性は生死にかかわるほどの差ではないのであろう。

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